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Title: The impact of Zero-valent Iron Nanoparticles upon Soil Microbial Communities is Context Dependent

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Abstract

Purpose Nanosized zero valent iron (nZVI) is an effective land remediation tool, but there remains little information regarding its impact upon and interactions with the soil microbial community.

Methods nZVI stabilised with sodium carboxymethyl cellulose was applied to soils of three contrasting textures and organic matter contents to determine impacts on soil microbial biomass, phenotypic (phospholipid fatty acid - PLFA), and functional (multiple substrate induced respiration – MSIR) profiles.

Results The nZVI significantly reduced microbial biomass by 29% but only where soil was amended with 5% straw. Effects of nZVI on MSIR profiles were only evident in the clay soils, and were independent of organic matter content. PLFA profiling indicated that the soil microbial community structure in sandy soils were apparently the most, and clay soils the least, vulnerable to nZVI suggesting a protective effect imparted by clays. Evidence of nZVI bactericidal effects on Gram negative bacteria and a potential reduction of Arbuscular Mycorrhizal fungi are presented.

Conclusion Data implies that the impact of nZVI on soil microbial communities is dependent on organic matter content and soil mineral type. Thereby evaluations of nZVI toxicity on soil microbial communities should consider context. The reduction of AM fungi following nZVI application may have implications for land remediation.

Keywords: *zero valent iron nanoparticles, soil, PLFA, microbial community, respiration.*

Abbreviations:

AM: Arbuscular Mycorrhizal fungi
BMR: Basal Metabolic Rate

CMC: Carboxy methylcellulose

FA: Fatty Acid

FAME: Fatty Acid Methyl Ester

MSIR: Multiple Substrate Induced Respiration

MUFA: Mono-unsaturated Fatty Acid

nZVI: nano-scale Vero Valent Iron

OM: Organic Matter

PC: Principal Component

PLFA: Phospholipid Fatty Acid

SEM: Scanning Electron Microscope

WRM: World Reference Base

Introduction

The reductive capabilities and high reactive surface area of nano-scale (typically <100nm in diameter) zero-valent iron (nZVI) renders it an effective remediation tool. As such, nZVI has been widely used to remediate soil and groundwater contamination by reductive transformation and detoxification of a wide range of contaminants such as trinitroglycerine (Saad et al. 2010), nitroaromatics (Bai et al. 2009), nitroamines (Naja et al. 2009), and trichloroethane (Wang et al. 2010). In situ, nZVI can quickly form aggregates and adhere to the surface of soils and sediments, thereby decreasing its reactivity and mobility. To aid delivery, nZVI particles are often stabilised against aggregation and agglomeration by dispersion on the surface with an inert polymer. Sodium carboxymethyl cellulose (CMC) stabilises Fe^0 nanoparticles by accelerating the nucleation of Fe^0 during the formulation of nZVI, and subsequently forms a bulky and negatively charged layer via sorption of CMC molecules onto the surface of nZVI (Naja et al. 2009; He and Zhao 2007). This CMC layer prevents nZVI from agglomerating through electrostatic stabilisation (He and Zhao 2007). Despite the growing use and effectiveness of stabilised nZVI for land remediation, the potential environmental risk is currently largely unknown (Grieger et al. 2010; Klaine et al. 2008). The unique properties of nZVI that make it an effective remediation tool ultimately lends novel properties that may affect soil biological processes (Brar et al. 2010). Consequently, the precautionary attitude in Europe limits the use of nZVI in remediation works, whereas in the USA this technology is extensively used (Mueller et al 2012).

It is widely recognised that microorganisms are critical for soil nutrient cycling processes, such as the decomposition of organic matter and the cycling of nutrients. To preserve such functions, it is important to understand the potential effects of engineered nanoparticles on soil microbial communities. There have been various reports of antibacterial effects of nanoparticles to microorganisms in vitro including: *Escherichia coli* (Li et al. 2009; Lee et al. 2008; Auffan et al. 2009), *Staphylococcus aureus* (Gordon et al. 2011), *Dehalococcoides* spp. (Auffan et al. 2009), and decreased enzyme activity (Shah et al. 2010). Conversely, nZVI stimulated methanogens (Xiu et al. 2010), sulphate reducers (Xiu et al. 2010; Kirschling et al. 2010) and total bacterial populations in contaminated aquifers (Kirschling et al. 2010). However, information regarding the impacts of engineered nanoparticles on soil microbial communities is currently limited (Dinesh et al. 2012) and appears conflicting. Current evidence demonstrates a minimal impact of fullerenes (C_{60}) (Tong et al. 2007; Johansen et al. 2008), but conversely clear toxicity effects have been reported for TiO_2 and ZnO (Du et al. 2011; Ge et al. 2011) on soil microbial parameters. Partitioning of C_{60} onto organic matter was the most likely factor

controlling bioavailability (Tong et al. 2007) and as such organic matter could negate the toxic effects of nanoparticles (Dinesh et al. 2012). Nano-scale silver has received some attention and effects include reduction of microbial biomass C, increased basal metabolic rate, and no effect on soil enzymes or microbial biomass N (Hänsch and Emmering 2010) and toxicity effects on microbial community's (Kumar et al. 2011). It is becoming evident that effects are specific to the nanoparticle selected. The only published research that observed the effects of zero-valent iron on soil microbial communities reported no negative impacts on enzyme activities (dehydrogenase, hydrolase, and ammonia oxidation potential) (Cullen et al 2011). However the authors acknowledged that the nZVI interfered with assay conditions and so conclusions are limited, and in addition only one soil texture (silt loam) was used. There remains little knowledge of how metal engineered nanoparticles act in the soil matrix, especially their adsorption onto clay minerals and onto the organic fraction (Dinesh et al. 2012).

Given that nZVI is a highly effective remediation tool, that there is a knowledge gap of the effects of nZVI on soil microbial communities, and the potential for nZVI toxicity to the soil microbial community, it is important provide information that may assist regulatory decision makers regarding its deployment. The aim of this present study was to determine how soil textural properties and organic matter influence the interaction of sodium carboxymethyl cellulose stabilised nZVI (hereafter referred to as nZVI) with the soil microbial community. The hypothesis was that nZVI added to soil would have an impact on both the compositional structure and functional capacity of the soil microbial community, and that that protective mechanisms may attenuate such effects by adsorption onto by clay and partitioning into soil organic matter.

Materials and methods

2.1 Preparation of sodium carboxymethyl cellulose stabilised nZVI

Detailed preparation procedures and descriptions of the nanoparticles produced are described by He and Zhao (2007). In brief, nZVI was manufactured by reducing Fe^{2+} ions (1.0 g l^{-1} of Fe) using a borohydride solution at a BH_4^-/Fe^{2+} molar ratio of 2 in a 0.2% CMC matrix under a nitrogen atmosphere to avoid Fe oxidation (Fig 1). The stabilised Fe suspension was stored under nitrogen and used within one hour of production. Spherical nanoparticles were obtained, with a mean particle size of 10 nm (±5.2 nm) measured using a Scanning Field Emission Gun SEM (FEI, model XL30).

2.2 Soil and experimental

Soils were taken from three arable areas, designated as sandy, loam and clay soil, were prescribed based on their differing textural properties (Table 1). The soils were obtained (Dutch style auger-depth 10cm) using the "W-of-best-fit" approach to ensure that the soils were representative of the location (Rowell 1994). Nine sample points at the nodes of the W were selected. Resultant soil samples were homogenised and passed through a 2 mm sieve. Aliquots (100g dry weight equivalent) of the soils were then amended with finely ground (using a pestle and mortar) straw (often incorporated into soils to improve organic matter) at rates of zero, 5% and 10% (calculated based on the soils fresh weight). Incorporation of organic matter in into the soils in this way represents the application of fresh organic matter to improve organic matter status. Soils were then preconditioned by incubation in 500ml polypropylene jars (QMX, UK) for four months in the dark at 15^{o}C and at 40% water holding capacity (n=90). The lids of the microcosm were left loose to allow gaseous exchange throughout the incubation period. Soil water content was maintained by adding sterile deionised water to replace evaporated water. Preconditioning allowed the microbial communities, the organic matter and mineral components to equilibrate. After the preconditioning period, 20ml of an nZVI was applied to half of the soils and 20 ml aliquots of deionised water added to the second half (control), for a full factorial (3 soil texture x 3 organic matter levels x 2 nZVI levels) experimental design. The sample was then thoroughly homogenised by hand-mixing. Five independent replicates of each treatment were established. Following the pre-conditioning period, the nZVI suspension was added to the soil drop-wise while mixing thoroughly and continuously to ensure that the nanoparticle suspension was mixed homogenously into the soil matrix. A similar method was employed by Ge et al. (2011). Deionised water was added to raise the water content of all samples to 100% of the water holding capacity, after which the samples were incubated for 2 weeks in the dark at 15 ^{o}C. Additions

of nZVI and water to the soil, and subsequent mixing, were performed aseptically. Application of nZVI at this rate was equivalent to 270 μg Fe g^{-1} dry weigh soil. This application rate is representative of that which would be added to soil in land remediation and similar to dosages utilised by other studies of the effects of nanopartices on soil microbial communities (Ge et al. 2011). Bringing the soils to 100% of their water holding capacity mimics deployment of nZVI in land remediation scenarios and additionally will minimise oxidation of the nZVI. Biological analysis (microbial biomass-C, phenotypic PLFA and functional respiration profiles) were performed on all samples after the four month incubation period.

2.3 Biological analyses

Microbial biomass-C was determined using the fumigation-extraction procedure (Jenkinson and Powlson 1976) using K_{EC} of 0.45 (Vance et al. 1987; Joergensen 1996). The soils' microbial community phenotypic characteristics were determined by PLFA analysis using a method was modified from Frostegård et al. (1993). Lipids were extracted from 7g freeze dried soil using the Bligh and Dyer (1959) ratio of 1:2:0.8 (v/v/v) of chloroform, methanol and citrate buffer. Extracted lipids were then fractionated by solid phase extraction. The phospholipid fraction was derivatised by mild alkaline methanolysis (Dowling et al. 1986). The resultant fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (6890N Agilent, USA) using G2070 ChemStation for G.C. systems software. FAMEs were separated using a HP-5 (Agilent Technologies) capillary column (30m length, 0.32 mm ID, 0.25 μm film) which is 5% phenylmethyl siloxane. The temperature program started at 50°C (1 min), to 160°C at 25°C/min, followed by 2°C/min to 240°C and 25°C/min to 310°C (10 min). The injector temperature was set at 310°C, Flame Ionization Detector set at 320°C, and He flow set at 1 ml/min. The resultant FAMEs were calculated as relative abundance (mol %). Identification was by comparison of sample retention time to a standard qualitative bacterial acid methyl ester mix (Supelco) and by using gas chromatography coupled with mass spectroscopy (Agilent, USA). The nomenclature of the fatty acids follows that of Tunlid and White (1992). The mol% of indicator fatty acids was used as an indicator of the presence of group of organisms. Indicator fatty acids included: 18:2ω6, 9 -ectomycorrhizal fungi (Kaiser et al. 2010; Frostegård and Bååth 1996), 16:1ω5-arbuscular mycorrhizal fungi (AM) (Olsson et al. 1999), the sum of *i*15:0, *ai*15:0, 15:0, 16:1, *i*16:0, 16:1ω9, 16:1ω7 t, *i*17:0, *ai*17:0, *cyc*-17:0, 17:0 and *cyc*-19:0- total bacteria (Frostegård and Bååth 1996), the sum of the *iso* and *anteiso* branched fatty acids *i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0- Gram-positive bacteria (Zelles 1999), the sum of 16:1, 16:1ω9, 16:1ω7c, 16:1ω7t, 16:1ω5, 21:1- Gram-negative bacteria (Zelles 1999), the ratio of 16:1ω7 *trans/cis* was used as an indicator of microbial stress, and the

fungal:bacterial ratio was calculated using the fungal biomarker (18:2ω6) divided by summed mol% of bacterial fatty acids (Frostegård and Bååth 1996). The FAME ratio of 16:1ω7 *trans/cis* has been used by various authors (Zelles 1999) but should be used with caution as its use has been criticised by Frostegård et al. (2011) as change may indicate a shift in species composition as well as indicate environmental stress. In addition Frostegård et al, (2011) suggested caution using 16:1ω5 as a signature fatty acid for AM fungi as it is also found in some bacterial species.

Functional characteristics were observed by multiple substrate respiration (MSIR) profiles based on the method of Degens and Harris (1997). This functional approach determines the short-term (<4h) ability of the soil communities to degrade a variety of carbon sources, and was shown to have high sensitivity to perturbation and results are interpretable in an ecologically meaningful way. CO_2 produced from soil amended with carbon substrate was determined according to Ritz et al. (2006). Substrates were added individually to each soil after the four month incubation period and subsequent CO_2 evolution monitored. The substrates added were D-glucose (75mM), L-arginine (15mM), α-ketoglutaric acid (9mM), citric acid (100mM), glutamine 15mM), and malic acid (100mM). Aliquots (500µl) of each substrate were added to 1g soil to ensure even distribution throughout the soil. Each soil's Basal Metabolic Rate was also determined according to Ritz (2006) by determining the rate of respiration without additional substrates. Carbon dioxide evolution was determined using the Rapid Automated Bacterial Impedance Technique (RABIT: Don Whitley Scientific, UK) as a respirometer. An alkaline solution was prepared which contained 0.5 % potassium hydroxide in 1.0 % molten agar (Oxoid agar No.1). Aliquots (1 ml) of the molten KOH/agar solution were added to each RABIT impedance tube. After cooling, the tubes were stoppered and agar stabilised at room temperature for at least 4h prior to use. The soil sample and substrate were combined in a separate glass boat such that the material was not in contact with either the impedance tube probes or the KOH/agar mix. The impedance cell containing the glass boats with the soil/substrate mix were then connected to the RABIT analyser in a horizontal position to maximise the surface area. Changes in conductivity (µS) were measured and quantified to CO_2 according to Ritz et al. (2006). Microbial respiratory response rates were determined over a four-hour incubation period at 25^{o}C.

2.4 Statistics

The PLFA and MSIR profile data was analysed similarly to Pawlett et al. (2009) by subjecting to principal component analysis (PCA) using the correlations matrix. Resultant factor scores and microbial biomass data was analysed by factorial ANOVA with post-hoc Fisher LSD with Statsoft, Inc. (2010) STATISTICA, version 9.1 stipulating a significance threshold of 5%.

Results

There were significant ($P<0.001$) soil textural class and organic matter amendment effects on microbial biomass (Table 2: Fig. 2). Additional treatment effects were imposed with a significant ($P<0.01$) nZVI x organic matter interaction which indicated that the effect of nZVI depended on the organic matter addition levels. Where 5% organic matter and nZVI were added to the soil together there was a 29% reduction of microbial biomass. There was no significant effect of nZVI on microbial biomass where there were no additions of organic matter, or where soil was amended with 10% organic matter.

The soil microbial communities phenotypic (PLFA) profiles altered significantly ($p<0.001$) with both textural classes and organic matter amendments (Table 2: Fig 3). PC1 and PC2 of the PCA together accounted for 48% of the total variation. nZVI caused additional shifts in phenotypic profiles as shown by shifts in both PC1 and PC2, with third order (soil texture x organic matter x nZVI) interaction effects ($p<0.01$). On PC1 the nZVI interaction effects were due to shifts in the microbial community of the sandy (none and 5% straw), loam (10% straw addition) and clay (5% straw) soils. On PC2 the nZVI interaction effects were on the sandy (10% straw) and loam (no organic matter added) soil. Fatty acids with a positive loading (>0.8) on PC1 included: 15:0, 16:0, 17:0, 18:2ω6, 9, and 18:1ω9t. Fatty acids with a negative loading (<-0.8) on PC1 included: *i*15:0, *ai*15:0, 16:0, *ai*16:0/16:1ω9, 16:1ω5, Me17:0, *cyc*17:0, *i*17:0, 17:1ω8, 17:0, 18:1 and 19:2. The only fatty acid influencing PC2 ordination was 18:0.

Treatment with nZVI did not have a significant ($P>0.05$) effect on the abundance of the fungal fatty acid, the sum of the Gram-positive *iso* and *anteiso* branched bacterial fatty acids, or the fatty acid indicator of microbial stress (16:1ω7 *trans/cis* ratio). However, there was a small but significant ($P<0.01$) increase (3.3%) in the sum of the total bacterial fatty acids and reduction (3.8%) of Gram-negative monounsaturated straight chain bacterial fatty acids. The increase of bacterial and reduction of Gram-negative fatty acids was independent of soil texture or organic matter interaction effects (Table 2 and 3).

There was a significant ($P<0.05$) reduction of the arbuscular mycorrhizal indicator fatty acid16:1ω5 following nZVI treatment. This reduction showed interaction effects with both soil texture and organic matter content (Table 2 and 3). For the sandy soil, the reduction was only significant where there were no organic matter additions, however in the clay the reduction was only significant where the soil was amended with 5% organic matter, and for the loam soil the reduction was significant where 5% and 10% organic matter was added, but not where there were no organic matter additions (Fig. 4).

The MSIR functional profile (Fig. 5) was distinct for each soil textural class and organic matter amendment level ($P<0.001$: Table 2). Combined, PC1 and PC2 of the PCA profile accounted for 84% of the total variation. There was a significant ($P<0.05$) soil texture x nZVI interaction effect on PC1. The soil texture nZVI interaction effect was due to an nZVI effect on clay soil only. Substrates that contributed to a negative loading (<-0.8) on PC1 included Citric Acid, α-ketoglutaric acid, Glutamine, Malic Acid, and L-Arginine. No substrates had a positive loading.

The soils BMR significantly ($P<0.002$: Table 2) increased at each level of organic matter addition, however there were no effects of either soil textural class or nZVI addition. The mean BMR was 1.1, 3.0 and 5.4 (± 0.3 pooled SE) mg CO_2-C g^{-1} h^{-1} for the three levels of organic amendment of no additional organic matter, 5% and 10% additions respectively.

Discussion

Soil texture and organic matter amendment were the overriding sources of experimental variation, with distinct phenotypic (PLFA) and functional (MSIR) community profiles, and microbial biomass. Additional nZVI treatment effects were observed, but with subtle effects with both soil texture and organic matter thereby implying that effects were significantly dependent on soil characteristics. The BMR increased with organic matter amendment, but neither soil textural class not nZVI affected BMR.

The reduction of microbial biomass in nZVI treated soils occurred irrespective of soil texture, but was influenced by organic matter content. Only the soil amended with 5% straw had reduced biomass. There were no effects where no straw or 10% straw was added. The reduction of microbial biomass in the 5% straw amendment is likely to be a bactericidal rather than fungicidal response as the indicator fatty acid for ectomycorrhizal fungal was unaffected. More specifically, PLFA data indicates that the reduction of microbial biomass was primarily due to reduced Gram-negative bacteria. Other researchers have reported antibacterial properties of TiO_2 and ZnO nanoparticles on soil microbial communities (Du et al. 2011; Ge et al. 2011). However application of TiO_2 and ZnO nanoparticles at rates similar to that used in this study (Ge et al. 2011) produced a substantially greater impact on soil microbiology, thereby suggesting nanoparticle specific toxicity effects. As would be expected, further increasing the organic matter to 10% increased the microbial biomass, however with this increased level of organic matter there was no nZVI effect. It appears that the effect of increasing the organic matter on the microbial biomass was greater than any of nZVI. Thereby there may be a threshold level beyond which the increase of soil microbial biomass due to organic matter addition is greater than that of nZVI's effect. Conversely, nZVI did not affect microbial biomass where there were no straw amendments, and hence low microbial biomass. This suggests that nZVI toxicity requires sufficient indigenous microbial biomass for its effects to become apparent.

The primary mechanism of nZVI toxicity is likely to be oxidative stress (Auffan et al. 2009; Klaine et al. 2008). Partitioning by adsorption of Fe nanoparticles into the organic matter fraction (Pédrot et al. 2011) is likely to affect their bioavailability (Tong et al. 2007; Pédrot et al. 2011). Where soil organic matter is high, there may be sufficient organic matter such that nZVI becomes partitioned into the organic matter (Tong et al. 2007) thereby reducing bactericidal effects. To demonstrate a threshold level of nZVI toxicity with increasing organic matter content it would be necessary to study further increments of organic matter. An alternative mechanism

may be that a portion of the microbial community was resistant to nZVI toxicity. With increased organic matter, the resistant species may have thrived thereby increasing microbial biomass.

The microbial community's phenotypic (PLFA) profiles of all soil textural classes responded to nZVI additions, however the influence of soil organic matter was inconsistent between soil textures. The sandy soil appears the most vulnerable to shifts in the phenotypic profile as all organic matter treatments demonstrated nZVI effects. In comparison, the loam responded to nZVI where no (PC2) and 10% straw (PC1) was added, and the clay soil where only 5% straw (PC1) was added. Thus the clay soil was the most resistant to shifts in phenotypic profiles. This indicates that clay may provide a protective mechanism against the influence of nZVI on the microbial community by iron adsorption onto clays and/or by preventing the effective movement of the nZVI within the clay structure. Joo et al. (2009) demonstrated that CMC coated TiO_2 nanoparticles were adsorbed on the surface of the soil mineral, a similar mechanism may occur for nZVI. The surface charge of both nanoparticles and soil minerals play an important role in their sorption behaviour (Jaisi and Elimelech 2009) and thereby their bioavailability.

The reduction of AM fungi may have implications in early-stage bio-remediation sites with low concentrations of plant available phosphate as plant growth and survival relies on the AM fungi symbiotic association. It is recognised that in the absence of plants within the experimental design there would be no carbon exchange from the plant to the AM fungi, and therefore the decomposition of AM fungi is likely to be accelerated irrespective of experimental variables. In addition it is also recognised that the signature fatty acid for AM fungi (16:1ω5) is also found in bacteria (Frostegård et al, 2011). However data suggests that the presence of nZVI further accelerated AM fungi reduction. This could be further confirmed by analysis of the neutral lipid fatty acid 16:1ω5.

Clay soil amended with 10% straw was the only soil that proved vulnerable to shift in the functional (catabolic) profiles following nZVI application. This treatment combination did not affect the phenotypic profile. It may be that since the shift in phenotypic composition was not sufficient to alter the functional component that the phenotypic composition would have sufficient capacity to recover over time.

Additional factors that are likely to affect the interactions between the nanoparticles and soil microbial community include dissolved organic molecules such as humic and fulvic acids as they can enhance the colloidal stability of nanomaterials (Peralta-Videa et al. 2011) and soil chemistry such as ionic strength and pH as they influence the balance between the free migration of particles and the deposition of the nanoparticles (Solovitch et al. 2010). It may be important to assess environmental impacts of nZVI on a case by case scenario to determine the risks and benefits associated with its use in land remediation

It was hypothesised that nZVI added to soil would have an impact on both the compositional structure and functional capacity of the soil microbial community and that clay and organic matter may attenuate such effects. Biomass data demonstrates a reduction of soil microbial biomass following nZVI addition that is dependent on organic matter composition but independent of soil textural properties. Effects of nZVI on the soil phenotypic profile are subtle, often with 3rd order interaction effects with soil texture and organic matter. Such interactions are difficult to interpret and indicate that nZVI effects are highly context dependent. However PLFA data suggests that the sandy soils are the most and clay soils least vulnerable to shifts in phenotypic profiles and as such may provide evidence of a protective mechanism for clays. In addition PLFA data provides evidence that Gram negative bacteria and AM fungi are sensitive to nZVI. The impact of nZVI on the functional profile was minimal, and was restricted to clay soil only, independent of organic matter. Thereby although clays provide protective mechanisms against shifts in phenotypic profiles they are more vulnerable to shifts in functional profiles. In addition, the lack of any effect of nZVI on the soils BMR suggests that the influence of soils metabolic processes was minimal.

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Figures

Fig. 1 SEM (Scanning Field Emission Gun, FEI, model XL30) image of freshly prepared nZVI particles with scale bars representing particle size.
Fig. 2 Organic matter amendment x nZVI interaction effect upon soil microbial biomass. Light grey bars represents no nZVI additions, dark grey represents nZVI additions. Bars denote means (n=15), whiskers denote pooled standard error. Bars labelled with different letters are significantly different ($P<0.05$) using Fisher LSD test.

Fig. 3 PCA Score plot of ordination means (n =5 ± standard error) showing the impact of nZVI on the PLFA community (phenotypic) profile with 3rd order (soil texture x organic matter amendment x nZVI) interaction effects. Arrows represent significant ($p<0.05$) effect of nZVI. *Circles* no straw added, *squares* 5% straw added, *triangles* 10% straw added. *Filled shape* nZVI added, *Open shape* no nZVI. Data are means; error bars represent pooled standard error.

Fig. 4 Arbuscular mycorrhizal fungi (16:1ω5) data showing groupings ($P>0.05$) indicated by letters a, b and c. Light grey bars represents no nZVI additions, dark grey represents nZVI additions. Organic matter ammendments are represented by 5% and 10%. Data are means; error bars represent pooled standard error.

Fig. 5 PCA score plot showing ordination means (n =15 ± standard error) of the microbial community's catabolic profile (MSIR) showing the soil texture x nZVI interaction effects. Arrows represent significant ($p<0.05$) effect of nZVI. *Circles* Sandy, *triangles* Loam, *squares* Clay. *Filled shape* nZVI added. Data are means; error bars represent pooled standard error.

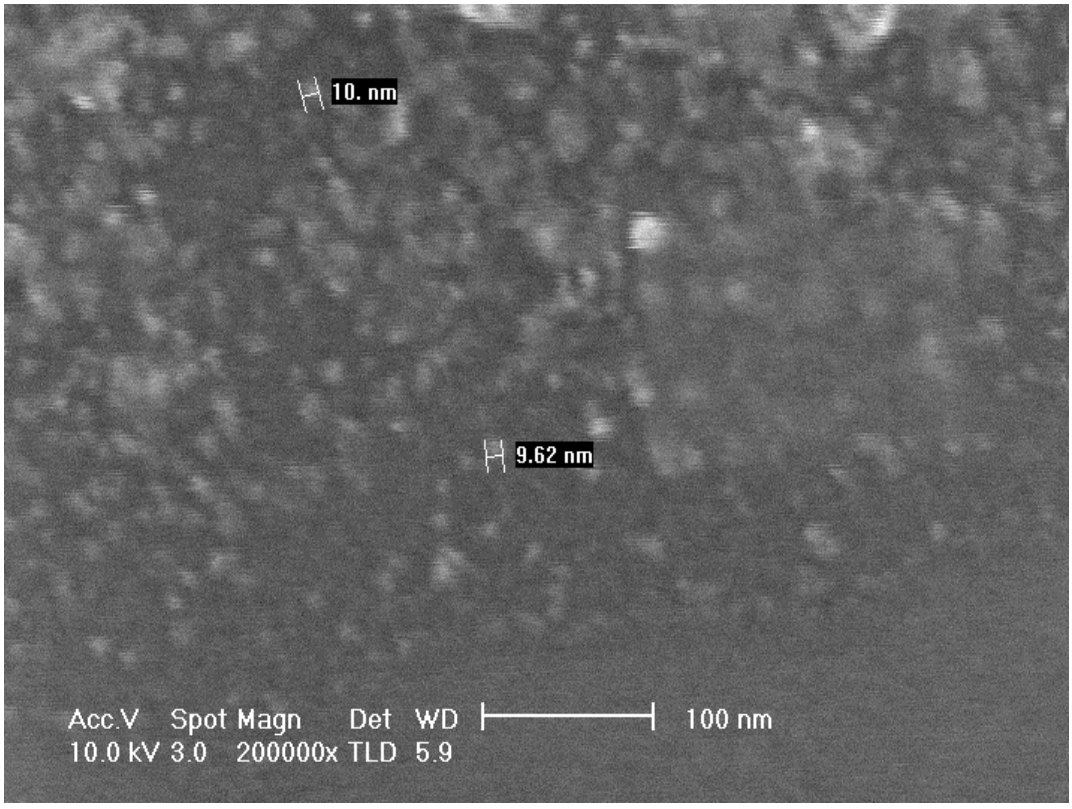

Fig. 1

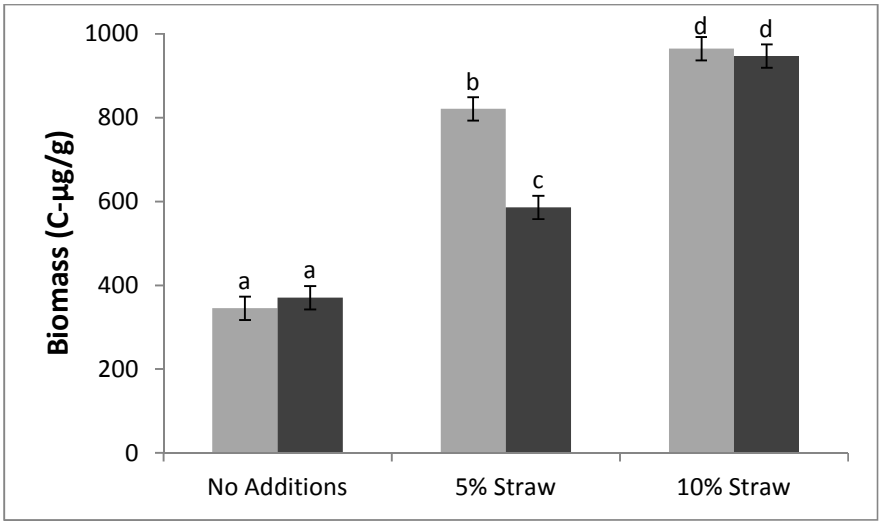
Biomass (C-µg/g)
1000
800
600
400
200
0
a
a
b
c
d
d
No Additions
5% Straw
10% Straw

Fig. 2

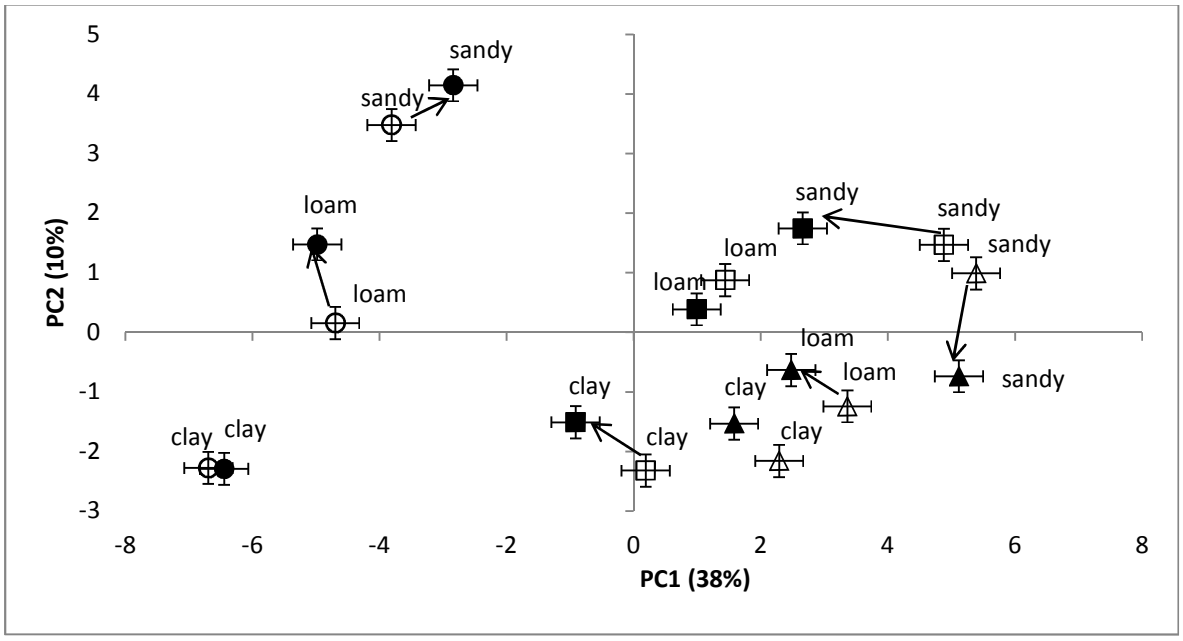
PC2 (10%)
PC1 (38%)
5
4
3
2
1
0
-1
-2
-3
-8
-6
-4
-2
0
2
4
6
8
sandy
sandy
loam
loam
clay
clay
clay
clay
sandy
sandy
sandy
loam
loam
loam
loam
clay
clay
clay
sandy

Fig. 3

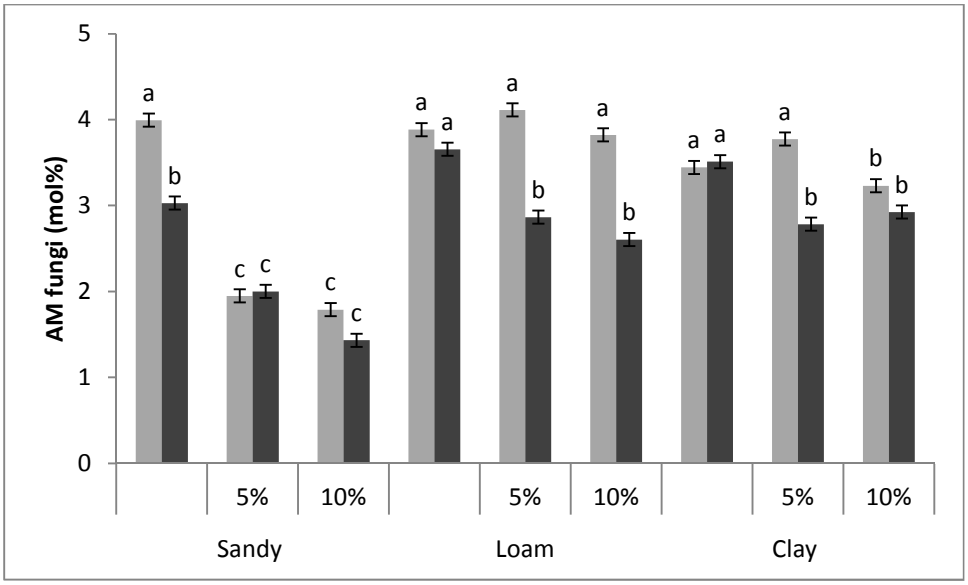
AM fungi (mol%)
5
4
3
2
1
0
a
b
c
c
c
c
a
a
a
b
a
b
a
a
a
b
b
5%
10%
5%
10%
5%
10%
Sandy
Loam
Clay

Fig. 4

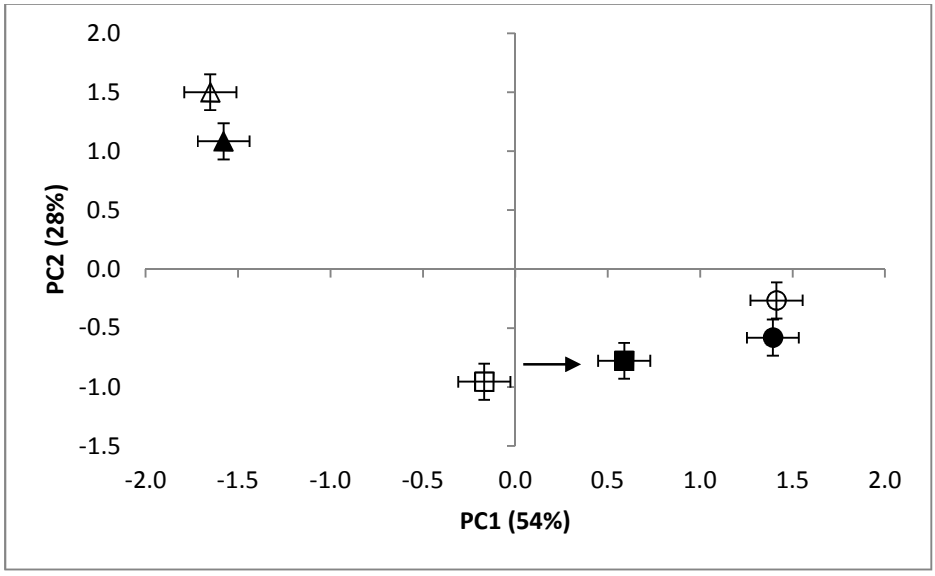
PC2 (28%)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
PC1 (54%)

Fig. 5

Tables

Table1: Description of the soil type and geographical location.

	Sandy	Loam	Clay
WRB reference	Arenosol	Cambisol	Stagnosol
Sub-group	Typical brown sand	Typical argillic pelosol	Pelostagnogleysol
Description	Sandy ferruginous (iron rich)	Clayey chalk drift	Clayey passing to clay or soft mudstone
Soil Series	Cottenham	Faulkbourne	Denchworth
Location	52º 00' 31" N 0º 26' 33" W	52º 00' 31" N 0º 26' 45" W	52º 00' 30" N 0º 26' 53" W
Elevation (m)	79.5	77.7	73.8
pH*$_a$	6.87	8.04	7.36
Total Carbon (%)*$_b$	1.20	3.20	3.43
Total Nitrogen (%)*$_b$	0.12	0.32	0.36
Sand (%)*$_3$	79.2	47.4	38.1
Silt (%)*$_c$	13.78	23.3	26.6
Clay (%)*$_c$	7.0	29.35	35.2
Textural class	Loamy Sand	Sandy Clay Loam	Clay Loam

[1]

[1] *N=3, WRB=World Reference Base, $_a$= ISO 10390:2005, $_b$= ISO 10694:1995, $_c$=ISO 11277:1998.

Table 2: ANOVA of microbial significance levels and variables.

	PLFA											MSIR	
	Microbial Biomass	BMR	PC1	PC2	Fungi (18:2ω6,9)	Bacterial FA	Fungi to bacterial FA ratio	Gram+ve FAs	MUFAs (Gram-)	*trans* to *cis* ratio of 16:1ω7	AM fungi (16:1ω5)	PC1	PC2
S	***	-	***	***	***	***	***	***	***	**	***	***	***
OM	***	***	***	***	***	***	***	***	***	-	***	***	***
ZVI	*	-	***	-	-	**	-	-	**	-	*	*	-
S x OM	**	-	*	***	-	*	-	-	-	*	***	-	-
S x ZVI	-	-	-	-	-	-	-	-	-	-	*	*	-
OM x ZVI	**	-	***	-	-	-	-	-	-	-	-	-	-
S x OM x ZVI	-	-	**	**	-	-	-	-	-	-	***	-	-

[2]

[2] BMR=Basal Metabolic Rate, S=soil texture, OM=organic matter; nZVI = zero valent iron with sodium carboxymethyl cellulose, PLFA=phospholipid fatty acid analysis, MSIR=multiple substrate induced respiration, PC=principal component, MUFA=monounsaturated fatty acids, AM=arbuscular mycorrhizal fungi. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, - no significant ($P>0.05$) effect

Table 3: Relative abundance of selected signature fatty acids, fatty acid ratios of microbial PLFA. Figures in brackets represent standard error.

Soil	Organic Matter (%)	nZVI	Bacterial FA	Fungi (18:2ω6,9)	Fungi to bacterial ratio	Gram+ FAs	MUFA (Gram-)	Trans to cis ratio (16:1ω7)	AM fungi
Sandy	0		35.2	2.3	0.07	18.9	10.4	31.8	4.0
			(0.2)	(0.1)	(0.00)	(0.2)	(0.2)	(5.3)	(0.2)
	0	+	34.7	2.3	0.07	18.8	9.6	26.0	3.0
			(1.1)	(0.1)	(0.01)	(1.4)	(0.3)	(3.7)	(0.1)
	5		30.3	9.9	0.33	16.0	8.3	27.9	1.9
			(1.2)	(0.9)	(0.03)	(1.0)	(0.5)	(2.8)	(0.2)
	5	+	33.2	9.9	0.30	18.0	8.1	18.7	2.0
			(0.4)	(0.7)	(0.02)	(0.5)	(0.2)	(0.6)	(0.1)
	10		29.4	14.1	0.49	15.7	7.3	30.5	1.8
			(1.3)	(1.1)	(0.06)	(1.6)	(0.2)	(3.4)	(0.2)
	10	+	30.4	14.4	0.47	13.9	7.7	22.1	1.4
			(0.5)	(0.8)	(0.03)	(0.3)	(0.2)	(1.9)	(0.0)
Loam	0		35.9	1.3	0.04	19.2	10.4	26.0	3.9
			(0.3)	(0.2)	(0.00)	(0.7)	(0.1)	(1.1)	(0.1)
	0	+	36.5	1.1	0.03	20.9	10.1	27.6	3.7
			(0.2)	(0.1)	(0.00)	(0.1)	(0.2)	(5.9)	(0.1)
	5		32.8	6.9	0.21	18.9	10.5	45.3	4.1
			(0.7)	(0.7)	(0.03)	(0.5)	(0.3)	(11.4)	(0.3)
	5	+	32.5	5.8	0.18	17.8	9.2	27.5	2.9
			(0.5)	(0.7)	(0.02)	(0.5)	(0.4)	(5.3)	(0.2)
	10		28.1	10.4	0.37	14.8	9.6	23.2	3.8
			(0.3)	(0.6)	(0.02)	(0.2)	(0.2)	(1.3)	(0.3)
	10	+	30.6	12.6	0.41	16.7	9.1	31.0	2.6
			(0.4)	(1.3)	(0.05)	(0.2)	(0.3)	(3.0)	(0.3)

Clay	0		38.6	1.2	0.03	21.7	9.2	17.4	3.4
			(0.2)	(0.1)	(0.00)	(0.0)	(0.1)	(1.8)	(0.0)
	0	+	39.3	0.9	0.02	22.1	9.5	19.6	3.5
			(0.5)	(0.1)	(0.00)	(0.5)	(0.2)	(1.1)	(0.1)
	5		33.7	7.2	0.21	18.0	9.9	20.0	3.8
			(0.5)	(0.3)	(0.01)	(0.5)	(0.2)	(1.4)	(0.1)
	5	+	35.1	7.2	0.20	19.0	9.1	23.6	2.8
			(0.3)	(0.4)	(0.01)	(0.2)	(0.2)	(2.9)	(0.1)
	10		30.8	11.8	0.38	16.1	9.1	27.5	3.2
			(0.4)	(0.4)	(0.02)	(0.1)	(0.4)	(3.8)	(0.2)
	10	+	32.0	12.2	0.38	17.0	9.1	26.6	2.9
			(0.4)	(0.7)	(0.03)	(0.2)	(0.2)	(2.5)	(0.1)

[3]

[3] nZVI = zero valent iron with sodium carboxymethyl cellulose, MUFA Monounsaturated fatty acids, FA fatty acids, AM arbuscular mycorhizal fungi, Gram+ *iso* and *anteiso* FAs